

De novo Sequencing

Assemble a genome of a genetically uncharacterized species
Efficiently characterize plasmids, phages or BACs

Introduction

De novo sequencing is the state-of-the-art method to gain first insights into the genomic structure of genetically yet uncharacterized organisms or genetic elements such as plasmids or bacterial artificial chromosomes (BACs). Initially,

the target DNA is fragmented and subsequently sequenced by next generation sequencing (NGS) methods. The resulting sequencing reads are *de novo* assembled to reconstruct the original structure of the analyzed genome or genetic

element. The assembly is the foundation for downstream analyses such as the prediction of genes and regulatory genetic features or cloning experiments and their validation.

Microsynth's Competences and Services

For *de novo* sequencing, Microsynth provides a one-stop service from experimental design to bioinformatics analysis (see **Figure 1**). Our workflow covers the assembly of plasmids, phages or BACs up to larger genomes of prokaryotes and eukaryotes. You can either outsource the whole process or only single steps to us.

Experimental Design

The gain and impact of any NGS project depends on its experimental design. The choice of appropriate sampling and DNA isolation methods are only two examples of important points to consider. Make use of our experience: Microsynth's NGS specialists are happy to assist you from the start.

DNA Isolation

The isolation of high quality DNA is a crucial step for *de novo* sequencing because DNA quality strongly influences library preparation efficiency. In cases where the target organism or genetic element is associated with a host, it is desirable to deplete host DNA to prevent losses in sequencing capacity and interferences with the assembly process. You can either perform the isolation yourself or outsource this critical step to us. Microsynth has extensive

experience in DNA isolation from various challenging organisms and tissues.

Library Preparation and Sequencing

The major step in library preparation is the enzymatic or mechanical fragmentation of the DNA. Depending on the study goals, different sequencing strategies are available to find the optimal trade-off between read length and coverage. Illumina sequencing

results in shorter read lengths (up to 2x300 bp) but high coverage, while costs per sequenced base are low. PacBio sequencing results in long reads (~20 kb) and a high read quality, however the coverage is lower and costs per sequenced base are higher. Our NGS specialists will be happy to assist you in finding the best strategy for your project.

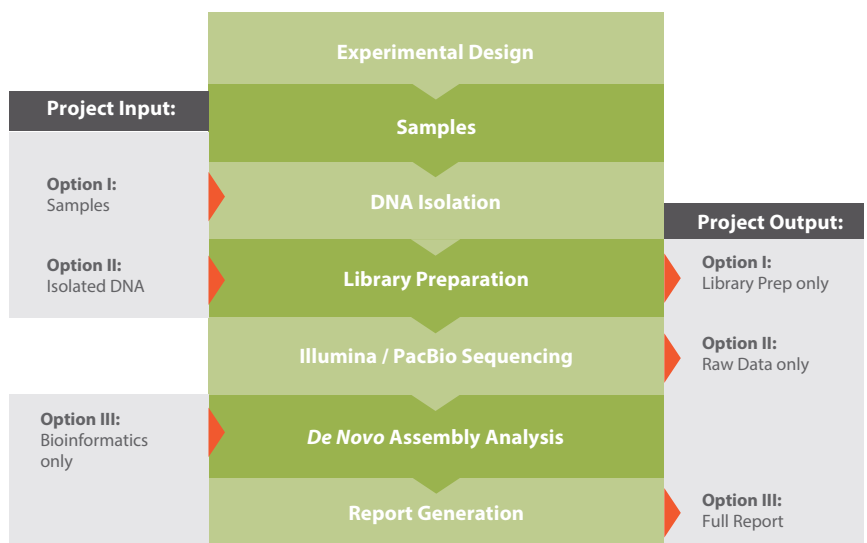


Figure 1. Microsynth's workflow for *de novo* sequencing projects. The workflow can be entered and exited at various steps dependent on the customer's requirements..

Bioinformatics Analysis

De novo assemblies are computationally challenging and many factors such as genome coverage, host contaminations or presence of repetitive structures have to be taken into account. After initial quality control, host associated reads are removed, if applicable, as

they could interfere with the assembly process. To get an optimal and evenly distributed coverage for the *de novo* assembly, sequencing reads are subsampled or normalized. For the *de novo* assembly, various assembling software are used, allowing both classical *de novo* approaches as well as reference guided

assemblies. We will select the optimal software and parameter set according to the requirements for your sample to produce the best possible assembly. The assembled contigs or scaffolds together with overview statistics make up the output of our bioinformatics analysis.

Example Results

Besides the assembled contigs or scaffolds in fasta format, we additionally

provide a variety of statistics including information on cumulative assembly

length and size distribution of the assembled contigs (see **Figure 2**).

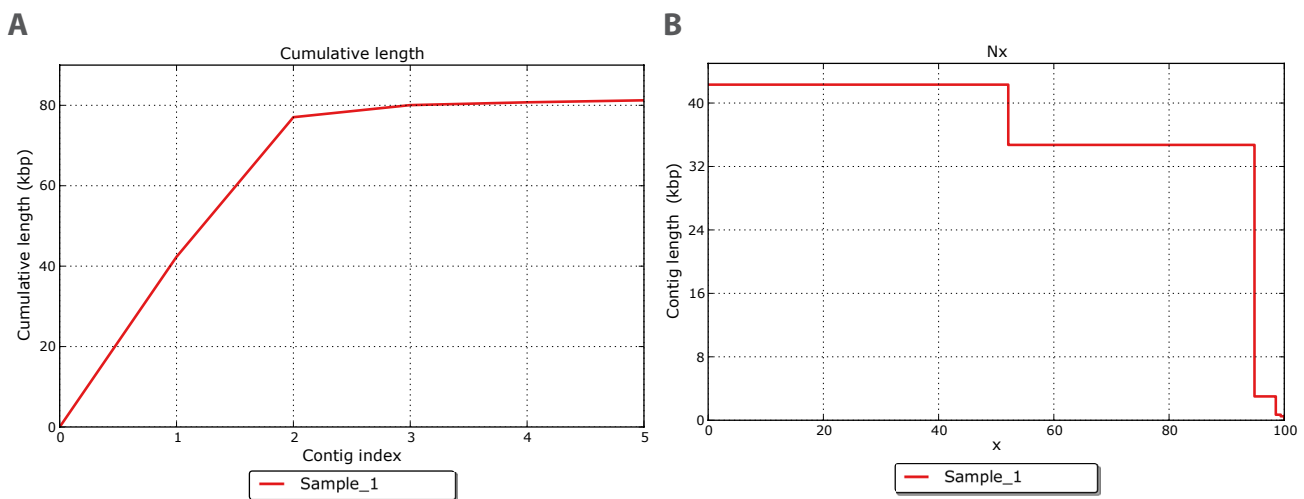


Figure 2. Graphical representation of assembly-related statistics 2A. Cumulative assembly length. 2B. Nx statistics for contig size.

Related Topics

For *de novo* information on previously unknown transcriptomes including annotation, please refer to our reference transcriptome sequencing service. If you are interested in the genetic variation of your specimen, our microbial or eukaryotic resequencing services offer an attractive solution.